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## CHAPTER

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# The Role of Interleukin-2 in Memory CD8 Cell Differentiation

Onur Boyman,\* Jae-Ho Cho and Jonathan Sprent

### Abstract

The current literature on the role of interleukin (IL)-2 in memory CD8<sup>+</sup> T-cell differentiation indicates a significant contribution of IL-2 during primary and also secondary expansion of CD8<sup>+</sup> T-cells. IL-2 seems to be responsible for optimal expansion and generation of effector functions following primary antigenic challenge. As the magnitude of T-cell expansion determines the numbers of memory CD8<sup>+</sup> T-cells surviving after pathogen elimination, these events influence memory cell generation. Moreover, during the contraction phase of an immune response where most antigen-specific CD8<sup>+</sup> T-cells disappear by apoptosis, IL-2 signals are able to rescue CD8<sup>+</sup> T-cells from cell death and provide a durable increase in memory CD8<sup>+</sup> T-cell counts. At the memory stage, CD8<sup>+</sup> T-cell frequencies can be boosted by administration of exogenous IL-2. Significantly, only CD8<sup>+</sup> T-cells that have received IL-2 signals during initial priming are able to mediate efficient secondary expansion following renewed antigenic challenge. Thus, IL-2 signals during different phases of an immune response are key in optimizing CD8<sup>+</sup> T-cell functions, thereby affecting both primary and secondary responses of these T-cells.

### Introduction

Typical T-cell receptor (TCR) $\alpha\beta$  T-cells are derived from precursors that migrate to the thymus where they undergo a series of selection and maturation processes termed positive and negative selection.<sup>1</sup> During positive selection, T-cells with newly-arranged TCR $\alpha\beta$  complexes are tested for their ability to receive survival signals via TCR contact with self-peptides bound to major histocompatibility complex (MHC) molecules expressed on cortical epithelium: cells with low but significant reactivity for self-peptide/MHC ligands are selected for survival while low-affinity cells die in situ, thus selecting only T-cells with functional TCRs. Conversely, through contact with antigen on bone marrow (BM)-derived dendritic cells (DCs), negative selection eliminates T-cells with high affinity for self-peptide/MHC molecules, thus ensuring the deletion of potentially auto-reactive T-cells. At the end of these selection processes, mature CD4<sup>+</sup> and CD8<sup>+</sup> T-cells are generated and subsequently released into the bloodstream for export to the secondary lymphoid organs. Despite the fact that these cells are selected on self-peptide/MHC molecules in the thymus, the cells have not yet seen foreign antigens and are thus immunologically naïve.

Post-thymic naïve T-cells recirculate continuously between blood and lymph through the lymphoid tissues and remain in interphase, rarely if ever dividing.<sup>2,3</sup> Activation of naïve T-cells occurs in the secondary lymphoid organs, such as lymph nodes and spleen, upon encounter with their cognate antigen in the form of peptides bound to MHC molecules presented by mature

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antigen-presenting cells (APCs) that express costimulatory molecules, notably DCs.<sup>4</sup> Upon activation, T-cells undergo vigorous clonal expansion and differentiate into effector cells which then home to the site of infection. These primed cells can directly exert their effector functions upon TCR engagement without the necessity for costimulatory signals; effector CD8<sup>+</sup> cells kill pathogen-infected cells whereas activated CD4<sup>+</sup> cells provide “help” for CD8<sup>+</sup> cell differentiation or induce B-cells to produce high-affinity antibodies.

At the end of the expansion of antigen-specific T-cells, which usually occurs after the pathogen has been eliminated, the immune response undergoes a contraction phase where most antigen-specific effector T-cells die via apoptosis.<sup>5,6</sup> However, a minority (about 5%) of antigen-specific T-cells survives to become long-lived memory cells.<sup>7,8</sup> These cells are resting cells but, unlike naïve T-cells, memory cells display certain surface markers (such as a high density of CD44 in mice) which distinguish these cells from naïve T-cells. Notably, unmanipulated normal mice contain significant numbers of cells with high expression of CD44 (CD44<sup>hi</sup>), thus closely resembling memory T-cells found after deliberate antigen priming.<sup>8,9</sup> These CD44<sup>hi</sup> “memory-phenotype” (MP) cells account for about 10-15% of total T-cells in young mice but become a majority population in old age; MP cells are thought to represent the descendants of T-cells reacting to ubiquitous environmental or self-antigens.<sup>10</sup>

Both naïve and memory T-cells are maintained in fairly stable numbers during normal steady-state conditions (reviewed in ref. 9). The homeostatic processes that govern T-cell survival are complex, but contact with two cytokines, IL-7 and IL-15, with or without TCR signals from contact with self-peptide/MHC ligands are of particular importance. For naïve T-cells, these cells are maintained through constant low-level signals via contact with IL-7 and self-peptide/MHC molecules (MHC-I for CD8<sup>+</sup> and MHC-II for CD4<sup>+</sup> cells).<sup>11,12</sup> For most memory (and MP) CD8<sup>+</sup> T-cells, by contrast, homeostasis depends on contact with both IL-7 and IL-15, while TCR contact with self-peptide/MHC-I ligands is relatively unimportant.<sup>13-15</sup> Similar to their CD8<sup>+</sup> counterparts, memory CD4<sup>+</sup> cells also require signals from IL-7 and IL-15 and do not depend on contact with MHC (MHC-II) molecules.<sup>16-18</sup> Typical memory and MP cells are resting cells which divide intermittently through contact with IL-15, the density of CD122, the receptor for IL-15, being higher on memory cells than naïve cells.<sup>19</sup> It should be mentioned that about one-third of MP cells are activated cells; these cells ignore cytokines and seem to be engaged in chronic responses to unknown self-peptide/MHC ligands, both for CD4<sup>+</sup> and CD8<sup>+</sup> cells.<sup>20,21</sup>

IL-7 and IL-15 belong to the family of common gamma chain ( $\gamma_c$ ) cytokines, which share usage of the  $\gamma_c$  receptor (also called CD132). This family also includes another cytokine that plays a central role in T-cell homeostasis, namely IL-2. This cytokine exerts complex effects on typical mature T-cells and is also primarily responsible for the survival of CD4<sup>+</sup> CD25<sup>+</sup> T regulatory cells (Tregs).<sup>22</sup> IL-2 is a 15 kDa short-chain four  $\alpha$ -helical bundle cytokine and is produced mainly by activated CD4<sup>+</sup> T helper cells, although activated CD8<sup>+</sup> T-cells, natural killer (NK) cells, NK T-cells and DCs stimulated with microbial products are also able to secrete IL-2, albeit in low amounts.<sup>23-29</sup> IL-2 acts in an autocrine or paracrine fashion by binding to IL-2 receptors (IL-2Rs).<sup>30</sup> High-affinity IL-2Rs are trimeric receptors consisting of IL-2R $\alpha$  (CD25), IL-2R $\beta$  (CD122) and the  $\gamma_c$  chain (Fig. 1); these receptors bind strongly to IL-2 with a dissociation constant ( $K_d$ ) of about  $10^{-11}$  M.<sup>31-33</sup> Trimeric IL-2Rs are found on Tregs as well as on recently-activated normal T-cells.<sup>30,34</sup> In addition to trimeric IL-2Rs, IL-2 can also bind to dimeric IL-2Rs consisting of CD122 and  $\gamma_c$ , albeit with a 100-fold lower affinity ( $K_d \sim 10^{-9}$  M). Dimeric IL-2Rs bind IL-15 in addition to IL-2 (Fig. 1) and are found at high levels on resting memory and MP CD8<sup>+</sup> cells as well as NK cells and at low but significant levels on naïve CD8<sup>+</sup> cells. Notably, CD122 and  $\gamma_c$  are responsible for mediating intracellular signaling whereas CD25 confers high-affinity binding to IL-2 but does not directly contribute to signal transduction.<sup>31</sup>

Below, we will review the role of IL-2 in CD8<sup>+</sup> T-cell responses *in vivo*; the *in vitro* actions of IL-2 have been reviewed extensively elsewhere.<sup>30-33</sup> In particular, we will discuss the contribution of IL-2 to the different phases of a CD8<sup>+</sup> T-cell response, starting with CD8<sup>+</sup> cell activation and expansion, followed by the contraction phase and then the memory phase. During each of these different stages, IL-2 has a decisive effect on CD8<sup>+</sup> cells. Thus, by controlling initial T-cell expansion

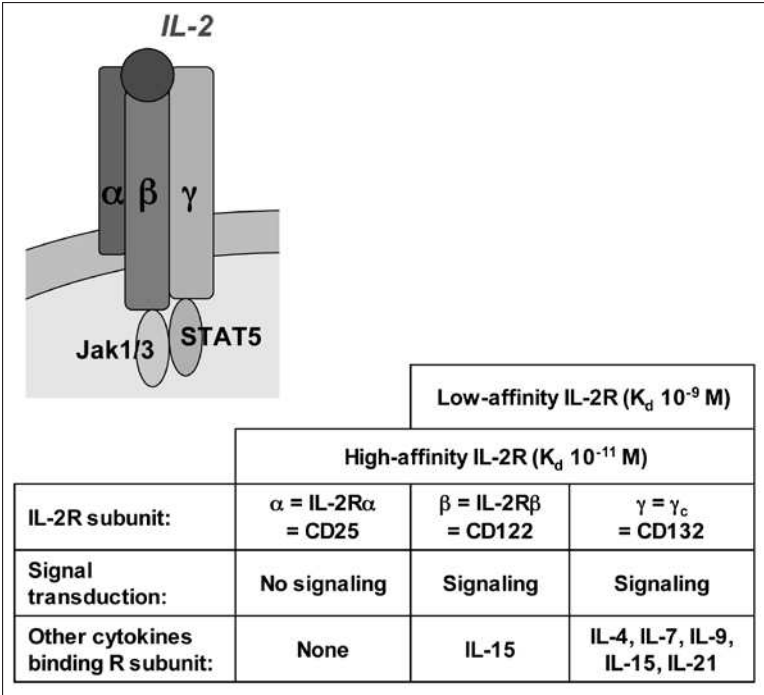


Figure 1. The IL-2 receptor (IL-2R) and its subunits. IL-2Rs are either dimeric IL-2R $\alpha\beta$  and bind IL-2 with a low affinity ( $K_d \sim 10^{-9}$  M) or trimeric IL-2R $\alpha\beta\gamma$  and associate with IL-2 with a  $K_d$  of about  $10^{-11}$  M. IL-2R $\alpha$  ( $\alpha$  subunit), also called CD25, is the private  $\alpha$  chain of IL-2 and does not bind any other cytokine. Moreover, CD25 does not contain a cytoplasmic tail and thus is not involved in signaling. Conversely, IL-2R $\beta$  ( $\beta$  subunit, also called CD122) and IL-2R $\gamma$  ( $\gamma$  subunit, also known as the common gamma chain,  $\gamma_c$ , or CD132) are crucial for signal transduction upon IL-2 binding to the IL-2R. CD122 is also a receptor subunit of the IL-15R, whereas  $\gamma_c$  is shared by all  $\gamma_c$  cytokines, i.e., IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Downstream signaling is mediated by the Jak-STAT pathway, notably involving Jak1, Jak3 and STAT5 as well as STAT3.

and differentiation during the primary response, IL-2 influences both the numbers and functions of the cells that survive to become long-lived memory cells.<sup>35</sup>

***IL-2 Signals During Priming Lead to Qualitative and Quantitative Differences in CD8<sup>+</sup> T-Cell Responses***

Once naïve T-cells encounter their cognate antigen presented by mature APCs and receive TCR and costimulatory signals, they become activated and begin to proliferate. Activation and proliferation of T-cells induces many changes, including the upregulation of CD25 and CD122, thus leading to expression of trimeric high-affinity IL-2Rs (Fig. 2). At the same time, activated T-cells, especially CD4<sup>+</sup> cells, start producing IL-2. Via synthesis of high-affinity IL-2Rs, activated T-cells, including CD8<sup>+</sup> cells, are highly sensitive to IL-2.

The contribution of IL-2 signals to primary CD8<sup>+</sup> T-cell responses has been studied using IL-2- or IL-2R-deficient mice (Table 1). Activation, expansion and primary effector functions of CD8<sup>+</sup> T-cells were tested in IL-2<sup>-/-</sup> mice following infection with lymphocytic choriomeningitis virus (LCMV) and vaccinia virus and also after peptide immunization or exposure to alloantigens.<sup>36-41</sup> The overall conclusion from these experiments is that functional immune responses do occur in

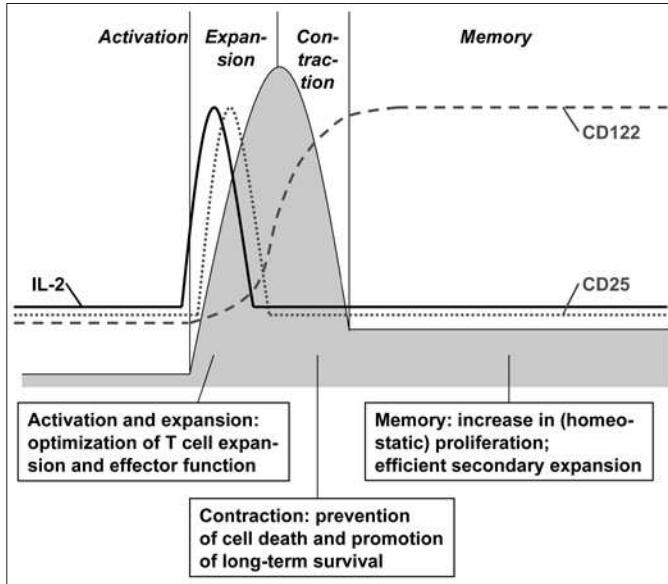


Figure 2. Expression levels of IL-2 and IL-2R subunits and the role of IL-2 during the different phases of a CD8<sup>+</sup> T-cell immune response. Following activation by a professional APC, naïve CD8<sup>+</sup> T-cells start to proliferate and expand. Proliferation is considerably enhanced by the concomitant production of IL-2 (solid line), which initially binds to low-affinity IL-2R $\alpha\beta$  receptors and, upon upregulation of CD25 (dotted line), to high-affinity IL-2R $\alpha\beta\gamma$  receptors. Notably, CD122 (dashed line) is also upregulated during expansion and is highest on memory T-cells, where it serves mainly for conferring responsiveness to IL-15. The role of endogenous IL-2 during activation and proliferation of CD8<sup>+</sup> T-cells is to optimize cell expansion; by contrast, contact with exogenous IL-2 during this phase does not seem to be necessary or beneficial. During the contraction phase, however, administration of exogenous (recombinant) IL-2 is highly beneficial in preventing cell death and thus allowing higher numbers of antigen-specific T-cells to survive as memory cells for several months. Endogenous IL-2 seems to be dispensable for CD8<sup>+</sup> T-cell survival during the contraction phase. For memory CD8<sup>+</sup> T-cells, homeostatic proliferation of these cells is augmented by the presence of endogenous IL-2 or the administration of exogenous IL-2.

IL-2<sup>-/-</sup> mice, but these responses are somewhat lower and less robust than in normal mice. The results are as follows; the shortcoming of the use of IL-2-deficient mice is discussed later.

In comparison to IL-2<sup>+/-</sup> heterozygous or wild-type (WT) control mice, IL-2<sup>-/-</sup> animals gave near-normal CD8<sup>+</sup> T-cell effector responses as measured by direct ex vivo cytotoxicity upon infection with vaccinia virus.<sup>36,37</sup> For infection with LCMV, a 3-fold decrease of cytotoxic T-lymphocyte (CTL) activity was noted in comparison to control mice when spleen cells from IL-2<sup>-/-</sup> mice were tested in a direct ex vivo cytotoxicity assay.<sup>36-39</sup> Moreover, spleen cells from IL-2<sup>-/-</sup> mice produced markedly reduced interferon (IFN)- $\gamma$  and IL-4 levels upon in vitro restimulation for 24 h.<sup>38,39</sup> Two studies concluded that these differences were not biologically significant because IL-2<sup>-/-</sup> mice infected with LCMV were as efficient as IL-2<sup>+/-</sup> or WT mice in mounting a delayed-type hypersensitivity response (as measure by footpad-swelling reaction), clearing LCMV below detection levels from spleens, livers and kidneys by days 9-10 and protection against lethal choriomeningitis after intracerebral infection with LCMV.<sup>36,37</sup> Conversely, others came to a different conclusion, reporting that IL-2<sup>-/-</sup> mice infected with LCMV contained detectable virus in the spleen and especially in the kidneys on day 7 after infection, whereas IL-2-competent control mice had cleared LCMV from these organs by that time.<sup>38</sup> These disparate findings may reflect the different doses of LCMV and routes of administration (300 plaque-forming units (PFU) intravenously vs. 2000 PFU intraperitoneally, see Table 1) used

in these studies. Whether this roughly 7-fold difference in initial viral load along with the dissimilar routes of infection could explain the differences observed remains to be tested.

Others found that IL-2 signals affected the expansion of antigen-specific CD8<sup>+</sup> T-cells only in nonlymphoid tissues (such as in lamina propria, epithelia, liver and lungs) but not in lymphoid organs such as the spleen. Thus, after adoptive transfer of IL-2<sup>-/-</sup> ovalbumin-specific OT-I TCR transgenic (tg) CD8<sup>+</sup> T-cells to IL-2<sup>-/-</sup> vs. WT mice followed by subsequent infection with recombinant vesicular stomatitis virus expressing ovalbumin, the authors found that paracrine IL-2 signals significantly increased the survival and sustained expansion of antigen-specific CD8<sup>+</sup> T-cells in nonlymphoid tissues but not in spleen; paradoxically, autocrine IL-2 signals (observed with WT tg cells) negatively influenced expansion in nonlymphoid tissues.<sup>42,43</sup> In another study, *Listeria monocytogenes* (LM)-specific TCR tg CD8<sup>+</sup> T-cells transferred to WT hosts underwent comparable expansion and production of IFN- $\gamma$  after infection with LM regardless of whether the donor antigen-specific CD8<sup>+</sup> T-cells were from an IL-2<sup>-/-</sup> or WT genetic background, thus providing further evidence that autocrine IL-2 was not essential for *in vivo* CD8<sup>+</sup> cell expansion and IFN- $\gamma$  production in response to LM.<sup>44</sup> However, the responding CD8<sup>+</sup> T-cells were still able to receive paracrine IL-2 signals in both situations.

Besides these above-mentioned studies on viral and bacterial infections, IL-2<sup>-/-</sup> mice were also used to test the role of IL-2 in CD8<sup>+</sup> responses to alloantigens. In one study, IL-2<sup>-/-</sup> vs. IL-2<sup>+/-</sup> and WT mice were rendered diabetic before transplantation with allogeneic islets grafts; allograft function was then followed by monitoring blood glucose measurements. The results showed that IL-2<sup>-/-</sup> mice were able to reject islet allografts, albeit with delayed kinetics compared to IL-2<sup>+/-</sup> or WT mice.<sup>40</sup> Similarly, in another study, vascularized cardiac allografts were rejected by IL-2<sup>-/-</sup> mice, though here rejection was as rapid as with WT mice.<sup>41</sup>

The contribution of IL-2 to CD8<sup>+</sup> T-cell responses has also been tested with the aid of CD25<sup>-/-</sup> mice. In the absence of CD25, IL-2 is able to bind to and signal by the dimeric IL-2R $\beta\gamma$  receptor,<sup>45,46</sup> even though such binding is around 100-fold weaker than to the trimeric high-affinity IL-2R $\alpha\beta\gamma$  complexes.<sup>31-33</sup> To examine the influence of CD25, CD25<sup>-/-</sup> vs. WT OT-I TCR tg CD8<sup>+</sup> T-cells were transferred to WT recipients, which then received either recombinant vesicular stomatitis virus expressing ovalbumin, soluble ovalbumin or tumor cells expressing ovalbumin. These experiments showed that IL-2 signaling through high-affinity IL-2Rs was not important for initial division of the responding CD8<sup>+</sup> cells, even though CD25 was upregulated on WT OT-I cells before the first division; however, IL-2 was necessary for optimal expansion and sustained survival of the responding cells.<sup>43</sup> Interestingly, CD8<sup>+</sup> tg T-cells engineered to be capable of prolonged IL-2R-mediated signaling showed a significant increase in expansion of CD8<sup>+</sup> cells in response to LCMV, followed by enhanced secondary responses upon re-exposure to antigen.<sup>47</sup>

Contrasting in part with these above-mentioned findings on IL-2, another study implicated an initial role for IL-15 in CD8<sup>+</sup> cell proliferation. This study examined polyclonal T-cell responses to alloantigens or superantigens *in vivo* and concluded that IL-15-driven initial cell division had to occur before IL-2 production; IL-2 synthesis and CD25 upregulation became evident towards the end of the T-cell expansion phase and IL-2 signaling during this stage decreased or even terminated T-cell proliferation via downregulation of the  $\gamma_c$  receptor.<sup>48</sup> For anti-viral responses, however, there is no evidence for downregulation of the  $\gamma_c$  receptor.<sup>49</sup> Moreover, administration of recombinant IL-2 (rIL-2) promotes the expansion of CD8<sup>+</sup> cells,<sup>43,49</sup> indicating that levels of  $\gamma_c$  receptors on the responding CD8<sup>+</sup> T-cells are sufficient for IL-2 signaling. These data thus question the notion of a negative role for IL-2 at the end of the T-cell expansion phase because of  $\gamma_c$  downregulation. T-cell expansion is probably curtailed largely through loss of contact with antigen at the end of the primary response, thus leading to a decrease in the stimulus for IL-2 production. However, a decline in IL-2 production may also involve other factors. Here, it is noteworthy that the factor B-lymphocyte-induced maturation protein 1 (BLIMP1), a transcriptional repressor, has been suggested to regulate terminal differentiation of effector T-cells by limiting IL-2 production and promoting activation-induced cell death (AICD).<sup>50-52</sup>



Table 1. Comparison of different studies examining the role of IL-2 during the different phases of CD8<sup>+</sup> T-cell immune responses

	Pathogen	Dose, Route	In Vivo Results and Outcome	Ref.
<b>a) Activation, expansion and effector function during the primary response</b>				
IL-2 <sup>-/-</sup>	VV WR	2 x 10 <sup>6</sup> PFU i.v.	No difference in ex vivo cytotoxic response	36,37
IL-2 <sup>-/-</sup>	LCMV WE or LCMV Arm	2 x 10 <sup>2</sup> PFU i.v. or 3 x 10 <sup>2</sup> PFU i.v.	3-fold decreased frequency after expansion but equal virus clearance from spleen, liver and kidneys	36,37
IL-2 <sup>-/-</sup>	LCMV Arm	2 x 10 <sup>4</sup> PFU i.p.	3-fold decreased frequency after expansion and reduced virus clearance from spleen and kidneys	38,39
OT-I IL-2 <sup>-/-</sup> or OT-I CD25 <sup>-/-</sup>	VSV-Ova Ova EL4/E.G7	1 x 10 <sup>6</sup> PFU i.v. 0.5-5 mg i.p. 5 x 10 <sup>6</sup> i.d.	Decreased expansion in nonlymphoid organs	42,43
IL-2 <sup>-/-</sup>	Alloantigen		Allograft rejection associated with delayed or similar kinetics	40,41
CD25 <sup>-/-</sup>	LCMV Arm or LM-Ova	2 x 10 <sup>5</sup> PFU i.p. or 3 x 10 <sup>3</sup> PFU i.v.	Equal or 5-fold reduced expansion of CD25 <sup>-/-</sup> CD8 <sup>+</sup> cells	46,56
CD122 <sup>-/-</sup>	LCMV Arm	2 x 10 <sup>3</sup> PFU i.v.	Defective primary CD8 <sup>+</sup> cell response as measured by footpad swelling	55
CD122 <sup>-/-</sup> tg	vJ5510 # Agonistic anti-CD3 mAb	5 x 10 <sup>7</sup> PFU i.p.	Signals through CD122 dispensable for generating CTL activity but necessary for optimal IFN- $\gamma$ production in vivo	57-59
rhlL-2*	LCMV Arm	2 x 10 <sup>5</sup> PFU i.p.	Equal expansion and virus clearance if rhlL-2 is given on days 0-8 after infection	49
rmIL-2	Alloantigen		Administration of IL-2 during first 4 d increases antigen-specific cell counts by 40%	60
IL-2	Superantigen		Prolonged expansion and survival of superantigen-specific T-cells	61
<b>b) Contraction phase</b>				
CD25 <sup>-/-</sup> #	LCMV Arm or LM-Ova	2 x 10 <sup>5</sup> PFU i.p. or 3 x 10 <sup>3</sup> PFU i.v.	Similar contraction of CD25 <sup>-/-</sup> and WT CD8 <sup>+</sup> cells	46,56

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Table 1. Continued

	Pathogen	Dose, Route	In Vivo Results and Outcome	Ref.
OT-I IL-2 <sup>-/-</sup>	VSV-Ova	1 x 10 <sup>6</sup> PFU i.v.	Decreased apoptosis of antigen-specific T-cells	42,59
rhIL-2*	LCMV Arm	2 x 10 <sup>5</sup> PFU i.p.	Decreased apoptosis and increased long-term survival if rhIL-2 is given on days 8-15	49
<b>c) Early and late memory, secondary responses</b>				
IL-2 <sup>-/-</sup>	VV WR	2 x 10 <sup>6</sup> PFU i.v.	Decreased secondary ex vivo cytotoxic response	36
IL-2 <sup>-/-</sup>	LCMV WE or LCMV Arm	2 x 10 <sup>3</sup> PFU i.v. or 3 x 10 <sup>2</sup> PFU i.v.	Equal footpad-swelling reaction and equal protection upon lethal challenge in vivo	36
CD25 <sup>-/-</sup> #	LCMV Arm or LM-Ova	2 x 10 <sup>5</sup> PFU i.p. or 3 x 10 <sup>3</sup> PFU i.v.	Impaired expansion of CD25 <sup>-/-</sup> memory CD8 <sup>+</sup> T-cells due to decreased IL-2 signals during priming	46,56
rhIL-2*	LCMV Arm	2 x 10 <sup>5</sup> PFU i.p.	Increased turnover and survival in IL-2-treated animals	49
<b>d) Homeostasis</b>				
CD25 <sup>-/-</sup> #	LCMV Arm	2 x 10 <sup>5</sup> PFU i.p.	Equal survival of WT and CD25 <sup>-/-</sup> CD8 <sup>+</sup> cells	46
rhIL-2*	LCMV Arm	2 x 10 <sup>5</sup> PFU i.p.	Increased turnover and survival of CD8 <sup>+</sup> cells in IL-2-treated animals	49

Abbreviations and explanations: \*: 15,000 international units (IU) rhIL-2 given twice daily; #: BM chimeras in which irradiated WT mice were reconstituted with T-cell-depleted BM from WT and CD25<sup>-/-</sup> mice; †: transgenic mice that express CD122 in thymocytes but lack CD122 expression in mature peripheral lymphocytes due to genetic deficiency; #: recombinant vaccinia virus expressing the spike protein S510 of the JHM strain of mouse hepatitis virus; VV WR: Vaccinia Virus strain Western Reserve; LCMV: lymphocytic choriomeningitis virus; WE: LCMV strain WE; Arm: LCMV strain Armstrong; Ova: ovalbumin; EL4 or E.G7: ovalbumin expressing tumor cell lines; VSV-Ova: recombinant vesicular stomatitis virus expressing ovalbumin; LM-Ova: recombinant *Listeria* monocytogenes expressing ovalbumin; PFU: plaque-forming units; i.v.: intravenous; i.p.: intraperitoneal; i.d.: intradermal; rhIL-2: recombinant human IL-2; rmlL-2: recombinant mouse IL-2.



It is important to note the shortcomings associated with the use of IL-2<sup>-/-</sup> and IL-2R<sup>-/-</sup> mice. In particular, these mice develop hyperplasia of secondary lymphoid organs along with a multi-organ inflammatory disease, thus partly obscuring other immune responses.<sup>53-55</sup> Moreover, T-cells developing in these animals do not receive any (or modified) IL-2 signals and develop in the absence of IL-2-dependent Tregs. In order to circumvent these problems, investigators have used a BM chimera approach where irradiated WT mice were reconstituted with a mixed population of T-cell-depleted BM from WT and CD25<sup>-/-</sup> mice, thus allowing a direct comparison of normal and CD25-deficient CD8<sup>+</sup> cell responses in a normal host. Subsequently, these mixed chimeras were infected with LCMV and virus-specific CD8<sup>+</sup> T-cells from WT or CD25<sup>-/-</sup> origin and analyzed based on differential expression of congenic markers. One group of researchers observed only minimal differences in the responses of the two populations of CD8<sup>+</sup> T-cells during primary expansion, regardless of whether polyclonal CD8<sup>+</sup> T-cells or LCMV-specific TCR tg CD8<sup>+</sup> T-cells were tested.<sup>46</sup> Conversely, also using LCMV infection (but another LCMV strain), others reported a 5-fold decrease in the primary expansion of CD25<sup>-/-</sup> polyclonal CD8<sup>+</sup> T-cells as compared to their WT counterparts.<sup>56</sup> IL-2 signals through high-affinity IL-2Rs might thus be important for maximal expansion of virus-specific CD8<sup>+</sup> T-cells. This finding is in line with the above data obtained using IL-2<sup>-/-</sup> mice.

Another approach for countering the severe pathology seen in IL-2<sup>-/-</sup> and IL-2R<sup>-/-</sup> mice is to limit IL-2 unresponsiveness selectively to peripheral T-cells but not thymocytes. This has been done by generating CD122<sup>-/-</sup> tg mice that express CD122 under the CD2 promoter, thus leading to selective expression in thymocytes;<sup>57</sup> these mice do not display pathology and show normal CD8<sup>+</sup> cell development. With these mice, it was shown that signals through CD122 (the common receptor for IL-2 and IL-15) were dispensable for generating expansion and CTL activity of CD8<sup>+</sup> T-cells following *in vivo* infection with recombinant vaccinia virus, injection of an agonistic anti-CD3 mAb or stimulation with superantigen.<sup>57,58</sup> Nevertheless, IFN- $\gamma$  production was somewhat reduced, indicating that optimal stimulation required signaling through CD122.<sup>57,58</sup> Others obtained similar findings by preparing OT-I TCR tg CD8<sup>+</sup> T-cells on a WT, CD122<sup>-/-</sup> or CD122<sup>-/-</sup> tg background and then transferring these cells to WT mice followed by administration of soluble ovalbumin; based on proliferation and generation of CTL activity *in vivo*, no significant difference was noted in antigen-specific CD8<sup>+</sup> T-cells from these different backgrounds.<sup>59</sup> These results with CD8<sup>+</sup> cells from CD122<sup>-/-</sup> tg mice contrast with the above data obtained by others using IL-2<sup>-/-</sup> and CD25<sup>-/-</sup> mice.

The various approaches described above were aimed at determining the role of endogenous IL-2 at normal physiological levels. The results of exposing CD8<sup>+</sup> cells to exogenous IL-2 are considered below.

The effects of administering low-dose recombinant human (rh) IL-2 during expansion of virus-specific CD8<sup>+</sup> T-cells was examined by giving mice two injections per day of 15,000 international units (IU) of rhIL-2 on days 0-8 after LCMV infection. Such IL-2 treatment resulted in similar LCMV-specific CD8<sup>+</sup> T-cell counts in IL-2-treated and control mice on day 8 after infection (though, surprisingly, numbers of virus-specific CD4<sup>+</sup> T-cells at the peak of the response were markedly reduced in IL-2-treated mice compared to controls).<sup>49</sup> Moreover, IL-2 treatment did not affect LCMV clearance from the spleen and viral titers became undetectable 9 days after infection in both groups. These results suggest that provision of additional IL-2 during the first 8 days following LCMV infection does not influence primary expansion and effector function of virus-specific CD8<sup>+</sup> T-cells (which contrasts with a negative influence on virus-specific CD4<sup>+</sup> cells). Interestingly, allospecific responses of 2C TCR tg CD8<sup>+</sup> T-cells to BALB/c (H-2<sup>d</sup>) splenocytes were found to be increased by about 40% following the administration of recombinant mouse (rm) IL-2 during the first 4 days of stimulation *in vivo*.<sup>60</sup> Moreover, for total T-cells stimulated with superantigens, implantation of an IL-2-containing osmotic pump prolonged the expansion and survival of superantigen-reactive T-cells as measured on day 6 after stimulation.<sup>61</sup> Thus, provision of exogenous IL-2 in these latter two settings might be beneficial because, under the conditions used, immune activation to alloantigens or superantigens was brief in the absence of IL-2 and therefore probably associated with much less proliferation than after virus infection. In the case of alloantigens, prolonged anti-host responses occur when T-cells are transferred to irradiated

H-2-different mice, thus eliciting graft-versus-host disease (GVHD). For GVHD produced by purified CD8<sup>+</sup> cells, disease induction is much worse when the hosts are injected repeatedly with rIL-2 starting at 1 week posttransfer or when donor CD8<sup>+</sup> cells are co-injected with IL-2-producing CD4<sup>+</sup> cells.<sup>62</sup> Paradoxically, with a mixed population of T-cells, administration of IL-2 from days 0-5 after T-cell transfer can protect against GVHD, perhaps by stimulating Tregs.<sup>63</sup>

Recently, it has been shown that naïve CD8<sup>+</sup> T-cells can proliferate vigorously and differentiate into MP cells when exposed to high levels of IL-2 *in vivo* in the absence of antigen. Such proliferation occurs when naïve CD8<sup>+</sup> cells are transferred to CD25<sup>-/-</sup> or CD122<sup>-/-</sup> mice; not being able to utilize IL-2, these mice have high levels of IL-2 and also IL-15 in the case of CD122<sup>-/-</sup> mice.<sup>64</sup> Antigen-independent proliferation of naïve CD8<sup>+</sup> cells to IL-2 also occurs after administration of rIL-2 mixed with a particular anti-IL-2 monoclonal antibody (mAb).<sup>45</sup> This combination leads to the formation of highly stimulatory IL-2/anti-IL-2 mAb complexes, which under *in vivo* conditions are able to stimulate polyclonal or TCR tg naïve CD8<sup>+</sup> T-cells to differentiate into effector cells able to produce IFN- $\gamma$ , tumor necrosis factor- $\alpha$  and granzyme B as well as lysis of target cells.<sup>64,65</sup> Subsequently, these IL-2/anti-IL-2 mAb complex-stimulated CD8<sup>+</sup> cells differentiated into MP cells (for polyclonal cells) or central memory cells (in the case of TCR tg cells). For TCR tg cells, OT-I memory CD8<sup>+</sup> cells generated by activation with IL-2/anti-IL-2 mAb complexes in the absence of antigen conferred efficient protection against challenge with recombinant LM expressing ovalbumin.<sup>65</sup> Notably, despite being antigen independent, this form of IL-2-driven proliferation of naïve CD8<sup>+</sup> cells was found to be highly dependent on contact with self-peptide/MHC-I molecules, i.e., as for naïve CD8<sup>+</sup> cells undergoing IL-7-driven homeostatic expansion. It should be noted that IL-2/mAb complexes also considerably enhance antigen-driven responses. Thus, when IL-2/anti-IL-2 mAb complexes were injected plus specific antigen to stimulate influenza-specific TCR transgenic CD8<sup>+</sup> T-cells *in vivo*, the complexes increased numbers of proliferating antigen-specific CD8<sup>+</sup> cells by 7-fold and conferred the cells with strong effector functions such as IFN- $\gamma$  production and CTL activity.<sup>66</sup>

### ***IL-2 and the Contraction Phase***

As mentioned earlier, most effector cells are eliminated at the end of the primary response, thus leading to a sharp contraction in total numbers of antigen-reactive cells. When BM chimeras containing a mixture of WT and CD25<sup>-/-</sup> cells were infected with LCMV, the decline in virus-specific CD8<sup>+</sup> cell numbers during the contraction phase was similar for WT and CD25<sup>-/-</sup> cells.<sup>46</sup> Thus, IL-2 signals via the high-affinity IL-2R do not seem to influence contraction. By contrast, injection of IL-2 during the contraction phase does prevent elimination of the responding cells. Thus, treating mice twice daily with 15,000 IU rhIL-2 on days 8-15 after LCMV infection resulted in a marked reduction of T-cell apoptosis and increased survival, both for CD8<sup>+</sup> and CD4<sup>+</sup> cells.<sup>49</sup> This effect was seen in both lymphoid and nonlymphoid organs (such as liver and lungs), indicating that IL-2 had a direct effect in promoting cell survival rather than causing an alteration in cell migration. Following this short course of IL-2 therapy for 1 week, elevated numbers of LCMV-specific CD8<sup>+</sup> and CD4<sup>+</sup> cells persisted for about 6 months before reaching the levels found in control mice. This potentiating effect of IL-2 on CD8<sup>+</sup> T-cell counts was not a byproduct of the increase in CD4<sup>+</sup> numbers but reflected a direct action of IL-2 on CD8<sup>+</sup> cells: thus, the beneficial effect of IL-2 on CD8<sup>+</sup> cell numbers also applied in mice lacking CD4<sup>+</sup> T-cells (either following depletion of CD4<sup>+</sup> T-cells by antibody in normal mice or by using CD4<sup>-/-</sup> mice).<sup>49</sup> On a per-cell basis, the CD8<sup>+</sup> cells from IL-2-treated mice were roughly as efficient as their counterparts from control animals. Collectively, these experiments suggest that IL-2 therapy during the contraction phase of an anti-viral immune response results in increased immunity to virus that lasts for several months (Fig. 2).

Similar findings on the beneficial role of exogenous IL-2 during the contraction phase were observed for the response of OT-I TCR tg CD8<sup>+</sup> T-cells to soluble ovalbumin or recombinant vesicular stomatitis virus expressing ovalbumin. Here, daily injections of IL-2 during the later stages of the primary response prevented elimination of the responding cells, although this effect lasted only for 1-2 weeks.<sup>43,59</sup>

### **Memory Cell Generation and Recall Responses**

During expansion and proliferation of antigen-specific T-cells a small subset of cells expresses high levels of IL-7R $\alpha$  (also called CD127); these cells give rise to long-lived memory cells, suggesting a role for IL-7 signals in the generation of functional memory cells.<sup>67</sup>

Recently, it was shown that IL-2 signals received during priming are necessary for efficient secondary responses of CD8<sup>+</sup> T-cell to viruses (Fig. 2).<sup>46,56</sup> Thus, for the above-mentioned mixed WT/CD25<sup>-/-</sup> BM chimeras, it was reported that LCMV-specific memory CD8<sup>+</sup> T-cells of CD25<sup>-/-</sup> origin expanded only 4-fold upon secondary antigen challenge 150 days after primary infection, compared to 40-fold expansion of WT memory CD8<sup>+</sup> cells.<sup>46</sup> Another group showed a 30- to 40-fold reduction in secondary expansion of CD25<sup>-/-</sup> antigen-specific CD8<sup>+</sup> T-cells, relative to WT cells, when measured 30-45 days after primary antigen challenge.<sup>56</sup> This defect was not due to impaired primary expansion or decreased homeostatic proliferation during the memory phase, although total numbers of virus-specific CD25<sup>-/-</sup> memory CD8<sup>+</sup> T-cells were 2- to 5-fold reduced in comparison to their WT counterparts. Instead, the failure to mount an efficient secondary response following challenge was due to a lack of IL-2 signals during priming. Thus, injection of IL-2 in the form of IL-2/anti-IL-2 mAb complexes<sup>45</sup> during the primary response allowed virus-specific CD25<sup>-/-</sup> memory CD8<sup>+</sup> T-cells to efficiently expand and survive following secondary antigen challenge;<sup>46</sup> injecting IL-2 during the secondary response, by contrast, was much less effective. Given that IL-2 acts through low-affinity IL-2Rs (CD122) on CD25<sup>-/-</sup> CD8<sup>+</sup> cells, it would be interesting to test whether enhanced levels of IL-15 could substitute for IL-2 in rescuing secondary responses of CD25<sup>-/-</sup> CD8<sup>+</sup> T-cells. In this respect, the poor generation of memory by CD25<sup>-/-</sup> CD8<sup>+</sup> T-cells also applies to normal CD8<sup>+</sup> cells primed in the absence of CD4<sup>+</sup> T-cells.<sup>68-70</sup> As CD4<sup>+</sup> T-cells are the main producers of IL-2 under steady-state conditions,<sup>25</sup> these findings consolidate the view that the optimal function of memory cells hinges on the precursors of these cells being exposed to IL-2 during initial priming. It should be mentioned that CD8<sup>+</sup> T-cells themselves can produce significant amounts of IL-2 in viral infections, but presumably in amounts insufficient to replace the need for IL-2 "help" from CD4<sup>+</sup> cells.<sup>71</sup>

With regard to resting memory CD8<sup>+</sup> cells, it was mentioned earlier that these cells divide sporadically through contact with background levels of IL-15. As for MP cells, the turnover of antigen (LCMV)-specific memory CD8<sup>+</sup> (and CD4<sup>+</sup>) cells is considerably enhanced following injection of exogenous IL-2 (or IL-15).<sup>49</sup> Since memory and MP cells have low expression levels of CD25, responsiveness of these cells to IL-2 (and IL-15) is mediated by low-affinity IL-2Rs.<sup>45</sup>

As for acute viral infection, IL-2 therapy can also lead to an increased frequency of virus-specific CD8<sup>+</sup> T-cells during chronic viral infections. Thus, when mice infected with LCMV clone 13, which results in a chronic infection, were treated with low-dose rhIL-2 for 1 week, LCMV-specific CD8<sup>+</sup> cell counts increased by about 10-fold and serum viral titers decreased in 80% of the animals.<sup>49</sup> This efficient stimulation of CD8<sup>+</sup> cells by IL-2 is somewhat surprising as CD8<sup>+</sup> T-cells found in chronic infections have only intermediate levels of CD122 and background levels of CD25 receptors,<sup>72</sup> which contrasts with the high levels of CD122 found on normal MP CD8<sup>+</sup> cells and memory CD8<sup>+</sup> T-cells generated after acute LCMV infection.<sup>19,73</sup> Notably, the LCMV-specific CD25<sup>-/-</sup> CD8<sup>+</sup> T-cells generated in mixed WT/CD25<sup>-/-</sup> BM chimeras declined rapidly during persistent viral infection.<sup>56</sup> Thus, for chronic viral infections, either exogenous or endogenous IL-2 signals seem to be beneficial or even crucial for the prolonged maintenance of the responding CD8<sup>+</sup> T-cells; these signals have to be delivered through high-affinity IL-2Rs.<sup>56</sup>

### **Memory Maintenance and Homeostasis**

As mentioned before, the few CD8<sup>+</sup> T-cells surviving the contraction phase and becoming resting memory cells are kept alive and in occasional cell division through contact with IL-15 and IL-7; these cells do not need TCR interaction with self-peptide/MHC-I molecules.<sup>13-15</sup> Currently, there is minimal evidence that IL-2 has a direct role in memory maintenance or homeostasis, probably because the background levels of IL-2 are too low to affect resting cells. Nevertheless, it is notable that antigen-specific CD8<sup>+</sup> memory cells generated in the absence of CD4<sup>+</sup> T-cells

gradually decrease during the memory phase.<sup>69,70</sup> This decrease might be due to a lack of CD4<sup>+</sup> cell-derived IL-2 signals. Interestingly, CD8<sup>+</sup> MP cells adoptively transferred to IL-2<sup>-/-</sup> recipients show a slightly reduced rate of homeostatic proliferation compared to WT recipients (O.B. and J.S., unpublished data). Hence, contact with endogenous IL-2 may play a significant, if minor role in memory CD8<sup>+</sup> cell homeostasis. As mentioned above, memory and MP CD8<sup>+</sup> cells are both strongly responsive to exogenous IL-2 signals.<sup>45,49</sup>

### ***Indirect Roles of IL-2 in the Generation of Memory CD8<sup>+</sup> T-Cells***

Through its role in controlling the survival of CD4<sup>+</sup> Tregs, IL-2 plays a vital role in maintaining immune tolerance.<sup>22,25,74,75</sup> Tregs, which are typified by high expression of CD25 and forkhead box p3 (Foxp3) transcription factor,<sup>34,76-78</sup> may impair memory cell generation indirectly by several mechanisms, including inhibiting the intensity of the primary response, secreting inhibitory cytokines and consuming stimulatory cytokines, including IL-2.<sup>79-83</sup> The many inhibitory functions of Tregs on the immune response have been discussed elsewhere.<sup>84-87</sup>

### **Concluding Comments**

In conclusion, IL-2 seems to have an important influence on CD8<sup>+</sup> cells at all stages of the immune response. Nevertheless, the evidence on this issue is still fragmentary and there are still substantial points of disagreement. For the primary response, most studies indicate that IL-2 is needed for optimal expansion and generation of effector functions. The discrepancies observed between individual studies may reflect the different systems used (pathogen vs. peptide stimulation) and/or differences in the precursor frequencies of antigen-specific CD8<sup>+</sup> T-cells: thus, systems with high frequencies of responding antigen-specific CD8<sup>+</sup> cells may consume large amounts of stimulatory cytokines, thus accentuating a need for IL-2 for optimal expansion. Alternatively, as suggested by some studies,<sup>43,48</sup> IL-2 might not be necessary for initial division of CD8<sup>+</sup> cells but rather for the late stages of proliferation, thus accentuating the overall magnitude of the primary response and thereby increasing the total numbers of effector cells available for differentiation into early memory cell precursors.<sup>35</sup> Since 90-95% of antigen-specific T-cells disappear by apoptosis during the contraction phase—perhaps largely because of loss of contact with growth factors—exposure to exogenous IL-2 during this stage can be highly beneficial in promoting cell survival and differentiation into early memory cells. Once these precursor cells upregulate CD122 and re-express IL-7R $\alpha$ , memory cells become sensitive to the stimulatory effects of IL-15 and IL-7, IL-15 inducing intermittent cell division and IL-7 controlling survival.<sup>9,10,19,45</sup> At this stage, administration of exogenous IL-2 is able to boost the frequency of memory T-cells, especially CD8<sup>+</sup> memory cells.<sup>45,49</sup> It is notable that IL-2 therapy is able to enhance proliferation of virus-specific CD8<sup>+</sup> T-cells during the contraction phase but not during initial expansion. Why exogenous IL-2 is generally ineffective during the expansion phase is unclear. A likely possibility is that, during this stage, the stimulatory effects of IL-2 are countered by various negative influences, including enhanced sensitivity to BLIMP1-mediated AICD and suppression through the activation of Tregs.<sup>51,52</sup> Overall, IL-2 signals can be viewed as fine-tuning the immune response, boosting weak responses and inhibiting excessive responses, thus promoting an optimal response that eliminates the pathogen concerned while maintaining normal self-tolerance.

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